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PATENT COOPERATION TREATY

PCT/EP2003/009101



Translation

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 0000054755	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/EP2003/009101	International filing date (day/month/year) 18 August 2003 (18.08.2003)	Priority date (day/month/year) 20 August 2002 (20.08.2002)
International Patent Classification (IPC) or national classification and IPC C12N 15/82		
Applicant SUNGENE GMBH & CO. KGAA		

<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of <u>8</u> sheets, including this cover sheet.</p> <p><input type="checkbox"/> This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of _____ sheets.</p>	
<p>3. This report contains indications relating to the following items:</p> <p>I <input checked="" type="checkbox"/> Basis of the report</p> <p>II <input type="checkbox"/> Priority</p> <p>III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p>IV <input checked="" type="checkbox"/> Lack of unity of invention</p> <p>V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p>VI <input type="checkbox"/> Certain documents cited</p> <p>VII <input type="checkbox"/> Certain defects in the international application</p> <p>VIII <input type="checkbox"/> Certain observations on the international application</p>	

Date of submission of the demand 23 January 2004 (23.01.2004)	Date of completion of this report 07 December 2004 (07.12.2004)
Name and mailing address of the IPEA/EP	Authorized officer
Facsimile No.	Telephone No.

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/EP2003/009101

## I. Basis of the report

### 1. With regard to the elements of the international application:\*

- ☐ the international application as originally filed
- ☒ the description:  
 pages \_\_\_\_\_ 1-79 \_\_\_\_\_, as originally filed  
 pages \_\_\_\_\_, filed with the demand  
 pages \_\_\_\_\_, filed with the letter of \_\_\_\_\_
- ☒ the claims:  
 pages \_\_\_\_\_ 1-43 \_\_\_\_\_, as originally filed  
 pages \_\_\_\_\_, as amended (together with any statement under Article 19  
 pages \_\_\_\_\_, filed with the demand  
 pages \_\_\_\_\_, filed with the letter of \_\_\_\_\_
- ☒ the drawings:  
 pages \_\_\_\_\_ 1/11 - 11/11 \_\_\_\_\_, as originally filed  
 pages \_\_\_\_\_, filed with the demand  
 pages \_\_\_\_\_, filed with the letter of \_\_\_\_\_
- ☒ the sequence listing part of the description:  
 pages \_\_\_\_\_ 1-42 \_\_\_\_\_, as originally filed  
 pages \_\_\_\_\_, filed with the demand  
 pages \_\_\_\_\_, filed with the letter of \_\_\_\_\_

### 2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item. These elements were available or furnished to this Authority in the following language \_\_\_\_\_ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

### 3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

### 4. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages \_\_\_\_\_
- ☐ the claims, Nos. \_\_\_\_\_
- ☐ the drawings, sheets/fig \_\_\_\_\_

### 5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).\*\*

\* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).

\*\* Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.

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**Supplemental Box**

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: IV.3

**Lack of unity of invention (PCT Article 34(3)(a))**

The International Preliminary Examining Authority agrees with the opinion of the International Searching Authority with regard to the lack of unity of the inventions (see ISR, sheet PCT/ISA/206). However, in the present case a written report can be established for all the inventions without substantial further effort. However, this assessment relates only to the establishment of the international preliminary examination report and is in no way binding in the event of examination in a national or regional phase.

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

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## V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

### 1. Statement

Novelty (N)	Claims		YES
	Claims	1-43	NO
Inventive step (IS)	Claims		YES
	Claims	1-43	NO
Industrial applicability (IA)	Claims	1-43	YES
	Claims		NO

### 2. Citations and explanations

Documents D1 and D2 were not cited in the intentional search report:

D1: WO99/55887 A (E.I. DU PONT DU NEMOURS AND COMPANY (US)) 4 November 1999 (1999-11-04)

D2: WO0008920 A (YISSUM RES DEV CO (IL))  
24 February 2000 (2000-02-24).

In addition, reference is made to the following documents, cited in the search report:

D3: WO01/88169 A (MONSANTO TECHNOLOGY LLC)  
22 November 2001 (2001-11-22)

D4: WO 00/32788 A (HANSENS LAB)  
8 June 2000 (2000-06-08)

D5: DHARMAPURI S ET AL: "Metabolic engineering of xanthophyll content in tomato fruits",  
FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS,  
AMSTERDAM, NL, vol. 519, nos. 1-3,  
22 May 2002 (2002-05-22), pages 30-34,  
XP004356816 ISSN: 0014-5793.

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1. Novelty and inventive step (PCT Article 33(2) and (3))

- 1.1 The applicant is advised that the document classified as a PX document in the search report was disregarded for the purpose of assessing novelty and inventive step in respect of the present claims, since it was assumed that the priority of the present application is valid and the priorities of the documents cited in the international search report have not been reviewed at the present time (cf. EPO Official Journal, 11/2001, pages 539-542; see in particular paragraph 13).
- 1.2 The application relates to methods for the production of  $\beta$ -carotinoids by cultivating genetically modified plants and to the use of these plants and an extract produced therefrom. Said methods are based on the overexpression of  $\beta$ -cyclase, optionally in combination with the overexpression of a hydroxylase or the suppression of  $\epsilon$ -cyclase and/or  $\beta$ -hydroxylase activity by the use of corresponding antisense, ribozyme, or cosuppression constructs.
- 1.3 D1 discloses, *inter alia*, the use of  $\beta$ -cyclase,  $\beta$ -hydroxylase and  $\epsilon$ -cyclase for producing transgenic plants with modified  $\alpha$ - or  $\beta$ -carotinoid content (figures 1-3, page 12, line 26 to page 14, line 2). In said document, the activity of the enzyme in question can be modulated by overexpression, preferably under the control of the endosperm-specific promoter zein, or by inhibition by

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cosuppression or antisense RNA. The inhibition of the activities of  $\beta$ -hydroxylase and  $\epsilon$ -cyclase in order to increase production of  $\beta$ -carotinoids or the  $\beta$ -carotinoid zeaxanthin is particularly emphasised therein (page 12, lines 31-39). Reference is also made to the use of plants that have been manipulated in this way as foodstuffs and animal feed or for the pigmentation of animal products. The amino acid sequence of the  $\beta$ -cyclase according to D1 (SEQ ID NO:12) has 66.1% sequence identity to the  $\beta$ -cyclase sequence (SEQ ID NO:2) of the present application. In consequence, the subject matter of the present claims 1-3, 5-24, 29-36 and 41-43 lacks novelty (PCT Article 33(2)). Furthermore, the subject matter of claims 4, 25-28 and 37-40 is not inventive (PCT Article 33(2) and (3)) since, for a person skilled in the art, the use of another known  $\beta$ -cyclase (e.g. see D2) is merely a routine variant of a method (analogue substitution) known *per se*. Similarly, the application of said methods to various members of different plant families is standard practice for a person skilled in the art and, in consequence, is not inventive.

- 1.4 D2 discloses a  $\beta$ -cyclase amino acid sequence from *Lycopersicon esculentum* (see SEQ ID NO:17 and SEQ ID NO:18, both of which have 100% sequence identity to the  $\beta$ -cyclase sequence SEQ ID NO:2 according to the application) and their use for the production of transgenic plants that have an increased  $\beta$ -carotinoid content in, in particular, chromogenic tissues such as fruits and flowers (page 4, lines 1-6; page 7, lines 5-7).

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Thus, the subject matter of claims 1-7, 23-32, 36, 37 and 39 lacks novelty and claims 38 and 40 do not involve an inventive step (PCT Article 33(2) and (3)) since, as set out in point 1.3 above, the use of the method in question in different plants is standard practice for a person skilled in the art.

- 1.5 D3 discloses, *inter alia*, methods for the production of  $\beta$ -carotinoids in plants by overexpressing  $\beta$ -cyclase or inhibiting  $\epsilon$ -cyclase by the expression of antisense DNA in photosynthetically inactive tissues (page 13, line 24 to page 14, line 5; page 3, lines 18-20). Furthermore, increasing the  $\beta$ -hydroxylase activity for the production of  $\beta$ -carotinoid zeaxanthin is described in D3. The  $\beta$ -cyclase from *Brassica napus* disclosed in D3 has a sequence identity of 51.9% to the SEQ ID NO:2 of the present application and, therefore, the present method claims and also the claims directed to genetically modified plants can be considered novel; however, claims 1-40 do not involve an inventive step (PCT Article 33(3)) since a person skilled in the art generally assumes that the heterological expression of an enzyme with comparable activity achieves an equivalent effect and enzymes of the same specificity can, if required, be interchanged. Furthermore, the uses of such genetically modified plants as foodstuffs and animal feed and the use of the extracts according to claims 41-42 are not inventive in the light of information found on page 3, line 24 to page 5, line 3; page 11, line 16 to page 12, line 2.

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1.6 D4 discloses methods for the production of different  $\beta$ -carotinoids by overexpressing or suppressing genes that code *inter alia* for  $\beta$ -cyclase,  $\epsilon$ -cyclase and  $\beta$ -hydroxylase (page 5, line 27 to page 6, line 24), the production of a specific carotinoid being controlled by the overexpression or suppression of one of said genes or of a plurality of genes in combination (page 6, lines 19-24). For the production of carotinoids, particular emphasis is laid on the overexpression of  $\beta$ -cyclase (page 20, lines 14-18) and the suppression of  $\epsilon$ -cyclase and  $\beta$ -hydroxylase (page 8, lines 32-34; page 17, lines 21-28). The simultaneous overexpression of  $\beta$ -cyclase and a hydroxylase is likewise mentioned (page 20, lines 20-25) and the use of transgenic flowers for producing foodstuffs and animal feed is also suggested (page 12, line 16 to page 13, line 2). Although the present claims 1-43 are novel over D4 (PCT Article 33(2)), they cannot be considered inventive (PCT Article 33(3)) since the amino acid sequence of the  $\beta$ -cyclase according to the invention has a sequence identity of 49% to the corresponding sequence according to D4.

1.7 D5 describes the overexpression of  $\beta$ -cyclase alone or in combination with  $\beta$ -hydroxylase under the control of a fruit-specific promoter (pds-promoter) in tomato plants. The  $\beta$ -cyclase used (from *Arabidopsis*) has 51.2% identity to the  $\beta$ -cyclase sequence SEQ ID NO:2 according to the present application.

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Thus, although the subject matter of claims 1-43 is novel in form, the subject matter of claims 1-14, 23-34 and 36-42 cannot be considered inventive relative to D5 since the overexpression of equivalent enzymes for achieving the same effect has already been disclosed in D5.

2. Further observations

2.1 Claims 11 and 12 relate to methods for the expression of a hydroxylase that is defined by means of a nucleic or amino acid sequence (SEQ ID NO:8 or SEQ.ID NO:9). However, the sequences defined in SEQ ID NO: 8 and SEQ ID NO:9 appear to code for an  $\epsilon$ -cyclase or a promotor thereof; SEQ ID NO:8 and SEQ.ID NO:9 show no sequence similarity to any hydroxylase known from the prior art. Thus, it is not clear for what method protection is sought in said claims (PCT Article 6).

2.2 Furthermore, the subject matter of claim 15 lacks clarity; claim 15 is *inter alia* dependent on claims 8-14, which relate to methods for **increasing hydroxylase activity** (and/or  $\beta$ -cyclase). However, claim 15, being an embodiment of a characterising feature, relates to a **reduced activity of the  $\beta$ -hydroxylase** (PCT Article 6).